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Recruitment of aphidophagous arthropods to sorghum plants infested with *Melanaphis sacchari* and *Schizaphis graminum* (Hemiptera: Aphididae)

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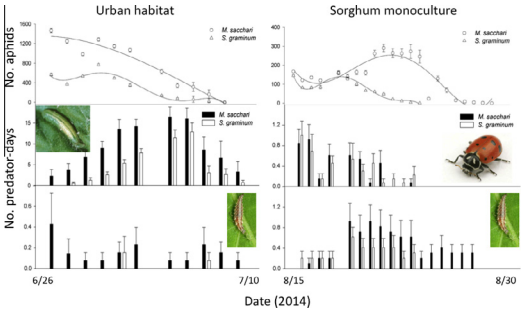
HIGHLIGHTS

- Sorghum infested with greenbug or sugarcane aphid recruited similar natural enemies.
- Syrphid larvae caused most mortality in open habitat with adjacent trees and flowers.
- Coccinellid adults caused most mortality in a sorghum monoculture with closed canopy.
- Chrysopids and aphelinids were secondary sources of mortality in both cohorts.
- Biological control was successful in preventing alate production by both species.

GRAPHICAL ABSTRACT



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ABSTRACT

A significant question in biological control is the extent to which indigenous natural enemies might be pre-adapted to exploit invasive species that constitute novel prey. We observed the recruitment of natural enemies to aphid microcosms – pots containing four sorghum plants infested with either *Melanaphis sacchari* (Zehntner), a newly invasive aphid, or *Schizaphis graminum* (Rondani), an established pest. The first cohort was monitored in open habitat along a tree line near riparian parkland and urban plantings, and the second, within a sorghum monoculture. Both aphid species were eliminated by natural enemies within 13 days in the first cohort, but in the second, *M. sacchari* reached higher numbers than *S. graminum* and survived a week longer. Biological control was successful in both cases; neither aphid produced a generation of alates, nor did plants sustain significant damage. Syrphid larvae, primarily *Allograpta obliqua* (Say), caused most aphid mortality in the first cohort, whereas adult Coccinellidae, primarily *Hippodamia convergens* Guerin-Meneville, caused most mortality in the second. Eggs and larvae of *Chrysoperla carnea* Stephens were present in both cohorts, but appeared to suffer more intraguild predation in the first. Flower flies and velvet mites were present only in the first cohort, and flower bugs, only in the second. *Aphelinus* sp. successfully parasitized both aphids, but *Lysiphlebus testaceipes* Cresson did not develop in *M. sacchari* due to their infection with the secondary endosymbiont *Hamiltonella defensa* (confirmed by DNA analysis). Thus, sorghum infested with *M. sacchari* attracted the same guild of natural enemies as *S. graminum* and had similar biological control outcomes. The findings suggest that the capacity of indigenous aphidophagous guilds to respond to, and ultimately control, invasive aphid species may be underestimated.

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1. Introduction

Invasions of exotic agricultural pests have become more frequent with increasing international air travel and the globalization of commerce. Economically damaging outbreaks of invasive pests typically occur during the first few years after their introduction, often leading some to the conclusion that exotic natural enemies will be required to provide biological control. The implicit assumption is that existing guilds of natural enemies will be insufficient, either because they lack specific adaptations to exploit the new pest, or because key niches are unoccupied (e.g., no specialized parasitoid is present). While this may be true in some cases, the preadaptations of many indigenous predators and parasitoids to utilize a new prey/host may be often underestimated, or these species may simply require a period of evolutionary adaptation to achieve their full potential as biological control agents. Aphids are a case in point, as they are vulnerable insects that feed in exposed locations and suffer attack from a broad guild of natural enemies. The taxa that are primarily or exclusively aphidophagous (e.g., Coccinellidae, Syrphidae, Chrysopidae, Braconidae, Aphelinidae) are ubiquitous in agroecosystems worldwide, even though local species composition varies. The present study was conducted to test the hypothesis that natural enemies of aphids in cereal crops on the High Plains possess substantial preadaptations for exploiting a novel aphid pest.

The sugarcane aphid, *Melanaphis sacchari* (Zehntner) (Hemiptera: Aphididae) is a cosmopolitan pest of sugarcane and sorghum capable of attacking a relatively broad range of host plants in the family Poaceae with economic impacts that vary from benign to devastating (Singh et al., 2004). Originally described as *Aphis sacchari* from specimens collected on sugarcane in Java, Indonesia (Zehntner, 1897), it was first reported in North America on sugarcane in Belle Glade, Florida in 1977 (Mead, 1978). Blackman and Eastop (2006) considered *Melanaphis sorghi* as a distinct species, but the morphological distinctions from *M. sacchari* are ambiguous and recent analyses of population genetics revealed clones defined by geography, rather than by host plant utilization (Nibouche et al., 2014). Thus *M. sorghi* is likely a synonym, as argued by Remaudiere and Remaudiere (1997), and *M. sacchari* appears to have arrived in the USA on infested sugarcane material from Hawaii (Nibouche et al., 2014).

The aphid became problematic on sugarcane in Louisiana soon after its detection in 1999 (White et al., 2001), but was not recorded infesting grain sorghum, *Sorghum bicolor* L., until the summer of 2013 in Beaumont, TX, during which highly damaging populations developed in fields throughout the Rio Grande Valley and across the border into Tamaulipas, Mexico (Villanueva et al., 2014). In 2014, *M. sacchari* range expansion occurred to the east and northeast, with sorghum infested in northern Texas, southern Oklahoma, Louisiana, Mississippi, Arkansas and Tennessee. To date, there have been no reports of the aphid west of Interstate 35, a north–south highway that bisects Texas and Oklahoma, but this may simply reflect the prevailing wind patterns during peak periods of aphid flight in 2014.

The primary feature of *M. sacchari* that contributes to its pest status on sorghum is a very high reproductive rate – more than double that of greenbug, *Schizaphis graminum* Rondani, on susceptible sorghum cultivars at 23–24 °C (FC, unpublished observations). Feeding by *M. sacchari* does not damage sorghum plants as quickly as feeding by greenbug, but uncontrolled colonies eventually cause similar chlorosis and death of plant tissues, although this requires a heavier load of aphids feeding for a longer period. Whereas *S. graminum* can feed within the panicle up until flowering and cause some flower sterility, seed weight and quality is usually unaffected, even though yields may be reduced (Harvey and Hackerott, 1974). In contrast, *M. sacchari* can continue feeding through the soft stages of grain fill, impacting both seed weight and quality (Chang and Fang, 1984; Berg et al., 2003). In addition, the thermal tolerance of this particular *M. sacchari* population has not yet been tested, but if it is capable of development and reproduction at temperatures exceeding 25 °C, this could contribute significantly to its pest status during hot summer conditions when high temperatures typically limit greenbug survival and reproduction (Pendleton et al., 2009; Michaud, in press). Another factor that could influence the pest status *M. sacchari* is its ability to utilize a wide range of wild and cultivated grasses, including barnyard grass, *Echinochloa crusgalli* (L.), Burmuda grass, *Cynodon dactylon* (L.) and Johnson grass, *Sorghum halepense* (L.) (Singh et al., 2004).

The literature suggests that a wide range of predators and parasitoids may contribute to biological control of *M. sacchari* throughout its geographic range. Singh et al. (2004) found 47 species of natural enemy reported to attack *M. sacchari*, with all major aphid natural enemy groups represented: Anthocoridae, Aphelinidae, Braconidae (Aphidiinae), Cecidomyiidae, Chamaemyiidae, Chrysopidae, Coccinellidae, Hemerobiidae, Lygaeidae, and Syrphidae. Anecdotal observations in 2014 indicate good initial recruitment of aphidophages to the first large *M. sacchari* infestations in south Texas (R. Villanueva, personal observations). However, there has been no effort yet to catalog the natural enemy species responding or to assess their rates of recruitment to *M. sacchari* in comparison to other aphids regularly infesting sorghum.

Over the past decade, our understanding of how natural enemies locate their herbivore prey by responding to induced plant volatiles has greatly improved (e.g., Takabayashi and Dicke, 1996; Arimura et al., 2005; Turlings and Ton, 2006). Adults of most aphid natural enemies orient to volatile compounds emitted by host plants in response to aphid feeding, (e.g., James et al., 2005; Sasso et al., 2009) and to odors of honeydew or aphid alarm pheromones (Hatano et al., 2008; Verheggen et al., 2008). Although many such compounds are ubiquitous across herbivore-plant associations, their activity is often dosage-dependent (e.g., Li et al., 2008). Furthermore, although variation among plant cultivars in emission profiles is well-recognized (e.g., Scutareanu et al., 2001; Kappers et al., 2011), the extent to which volatile profiles may vary among plants infested with different aphid species is not yet known. If aphid natural enemies must evolve responses to novel signals following new aphid-host plant associations, this could explain the delay in establishment of biological control when aphids are newly invasive in a region. Notwithstanding this, indigenous aphidophagous guilds typically deliver biological control of invasive aphids in time, although this may only be recognized when introduced exotic natural enemies either fail to establish, or have little impact (Michaud, 2002).

With the above considerations in mind, we designed a field experiment to compare the abundance and diversity of aphidophagous species recruited to potted plants of grain sorghum infested with either *M. sacchari* or *S. graminum*. We reasoned that, if *M. sacchari* infestation of sorghum elicits release of a volatile blend similar to that elicited by greenbug infestation, then the diversity and abundance of natural enemies attracted should be similar. However, if there are significant differences in recruitment of some species, but not others, it would suggest that different species may respond to different fractions of the volatile profile. Given that *M. sacchari* was not yet present in the study locality, the results provide an estimate of the extent to which aphidophagous insects on the High Plains are preadapted to discover and exploit *M. sacchari* on sorghum, and whether or not we can expect to eventually obtain levels of conservation biological control similar to those currently established for greenbug on this crop.

2. Materials and methods

2.1. Insect colonies

A colony of *S. graminum* 'biotype I', was established from material collected from sorghum in Hays, Kansas in 2013, whereas the *M. sacchari* colony was established from material obtained from the USDA-ARS laboratory in Stillwater, OK under a Material Transfer Agreement dated 27 May, 2014. Both species of aphids were reared on sorghum seedlings cultivar P85Y40 (Pioneer Hi-Bred, Johnston, IA), which has no specific aphid-resistant traits. Seedlings were grown in dense rows in metal trays (60.0 × 45.0 × 8.0 cm) in a greenhouse under natural light and infested by dislodging aphids from infested plants onto the new tray. Once infested, trays were transferred to climate-controlled growth chambers set to 23.0 ± 1.0 °C under continuous light.

2.2. Experimental setup

Experiments were conducted during the summer of 2014 at the K-State Agricultural Research Station-Hays in Hays, KS (38°51′N, 99°20′W). To produce plants for the experiment, seeds of P85Y40 sorghum were planted in plastic flower pots (35.0 cm diam) filled with soil, about 10 seeds per pot. The pots were placed in a greenhouse under natural lighting at an ambient temperature of 24.0–26.0 °C and watered daily. Shortly after seedling emergence, plants were thinned to leave four per pot and pots were moved outdoors to an exposed location in full sunlight to ensure exposure to wind and rain under natural conditions. This was essential for the normal development of plants robust enough to sustain aphid infestation under outdoor conditions.

Once plants reached the whorl stage (30.0–40.0 cm all), pots were returned to the greenhouse to be infestated with aphids. This was accomplished by clipping infested plants from the stock colonies of each aphid species and draping them over leaves of the potted plants. All plants were examined after 24 h and additional aphids were transferred, as needed, to ensure each plant had between 100 and 200 live aphids feeding. Each pot of four infested plants constituted a unit of sampling, hereafter refered to as a 'microcosm'. After 48 h, all pots were transported to the field where they were placed in a line a minimum of 10 meters apart, with aphid species alternating. Two cohorts of aphid microcosms were followed; the first consisting of 26 pots (n = 13 of each aphid species), and the second consisting of 28 pots (n = 14 of each species). The first cohort was established in the field on 25 June and was distributed along the northern edge of a coniferous tree line planted east–west in proximity to urban plantscapes, flower beds, mowed turf, and riparian parkland. This site was selected so that all pots would receive some afternoon shade and a degree of physical shelter, due to concerns about possible aphid mortality due to severe wind and rain events.

In contrast, the second cohort was established on 6 August in the middle of a 10 ha monoculture of forage sorghum with good herbicidal weed control, bordered by fields of mowed turf and wheat stubble. All pots were placed at least 15 m from the field border with the wheat stubble, with microcosms alternating in a north-west line in parallel with planted rows. In this case, the pots were partially dug in so that microcosm plants were similar in height to field plants at the start of observations and formed part of a continuous plant canopy. All pots were examined daily and watered as needed, with the exception of one day in each cohort when overnight rains made conditions too wet to count insects. On each observation day, the number of aphids on each plant was estimated and all eggs, larvae and adults of aphidophagous species were recorded. Because repeated observations of the same plants on successive days lead to many of the same insects being counted repeatedly, the only unbiased estimate of net 'predator presence' is provided by the sum of observations of each life stage of each species over all observation days, a measure expressed in 'arthropod life stage days'. Pupae that formed on plants (and some late instar syrphid larvae) were collected and held in a growth chamber in the laboratory at 23 °C until emergence of adults so that species identity could be confirmed. Whenever an aphid colony was eliminated, the plant was destructively sampled to locate all remaining predator larvae and pupae, as these often occurred in concealed sites within the whorl or behind leaf sheaths.

2.3. Statistical analysis

Arthropod counts were tallied as 'arthropod life stage days' on a per-pot basis, with the microcosm (group of four plants in one pot) as the experimental unit. Changes in the number of aphids per microcosm over time were analyzed graphically using polynomial regression and trend lines were fit using the equation that yielded the most significant parameters and the highest r^2 value. Fifth order equations provided lines of best fit in all cases except for *M. sacchari* microcosms in the first cohort, which were best described by a cubic function. Abundant natural enemy life stages were compared between aphid microcosms using repeated measures ANOVA and their daily means (±SE) are reported on a per-pot basis. When data was not normally distributed, it was subjected a Wilcoxon signed-rank test (SAS Institute, 2001) to test for effects of aphid species. The total number of observations of each life stage of each natural enemy species are reported separately for each aphid type in each cohort.

3. Results

In the first cohort, the last surviving microcosms of both aphid species effectively went extinct on the same day, but microcosms of *M. sacchari* in the second cohort persisted about a week longer than did those of *S. graminum* (Figs. 1A and 2A). Equations providing best fit to the data were as follows:

M. sacchari: $Y = 0.6X^3 - 17.8X^2 + 18.4X + 1346.4$, ($r^2 = 0.943$)
S. graminum: $Y = -0.2X^5 + 8.2X^4 + 95.6X^3 + 470.2X^2 - 930.1X + 1079.5$; $r^2 = 0.920$

The most abundant predators in the first cohort were syrphids (Table 1, Fig. 1B); of the 24 syrphid larvae and pupae reared out in the laboratory, 18 emerged as adults, of which 88.9% were *Allograpta obliqua* (Say) and 11.1% were *Syrphus* sp. Chrysopids were the next most abundant predator group and were assumed to be exclusively *Chrysoperla carnea* Stephens (Neuroptera: Chrysopidae), the only species normally collected in local grain crops. Larvae of these predators were directly observed consuming both aphid species on multiple occasions. There was no significant difference between aphid species in total numbers of syrphid egg days ($F = 0.83$; df = 1, 24; $P = 0.371$), but microcosms of *M. sacchari* tallied more syrphid larval days ($F = 13.29$; df = 1, 24; $P = 0.001$) and three times as many chrysopid egg days ($Z = 2.13$; $P = 0.033$) compared to those of *S. graminum*.

In the second cohort, adult Coccinellid were the most abundant predators, followed by *C. carnea* and *Orius insidiosus* (Say) (Hemiptera: Anthocoridae) (Figs. 1B and 2B). Equations providing best fit to the data were as follows:

M. sacchari: $Y = 6.5X^5 - 5.6X^4 + 0.0.86X48X^3 + 16.6X^2 - 89.8X + 249.5$; $r^2 = 0.832$
S. graminum: $Y = -0.02X^5 + 0.86X^4 - 13.66X^3 + 94.34X^2 - 265.48X + 329.41$; $r^2 = 0.947$

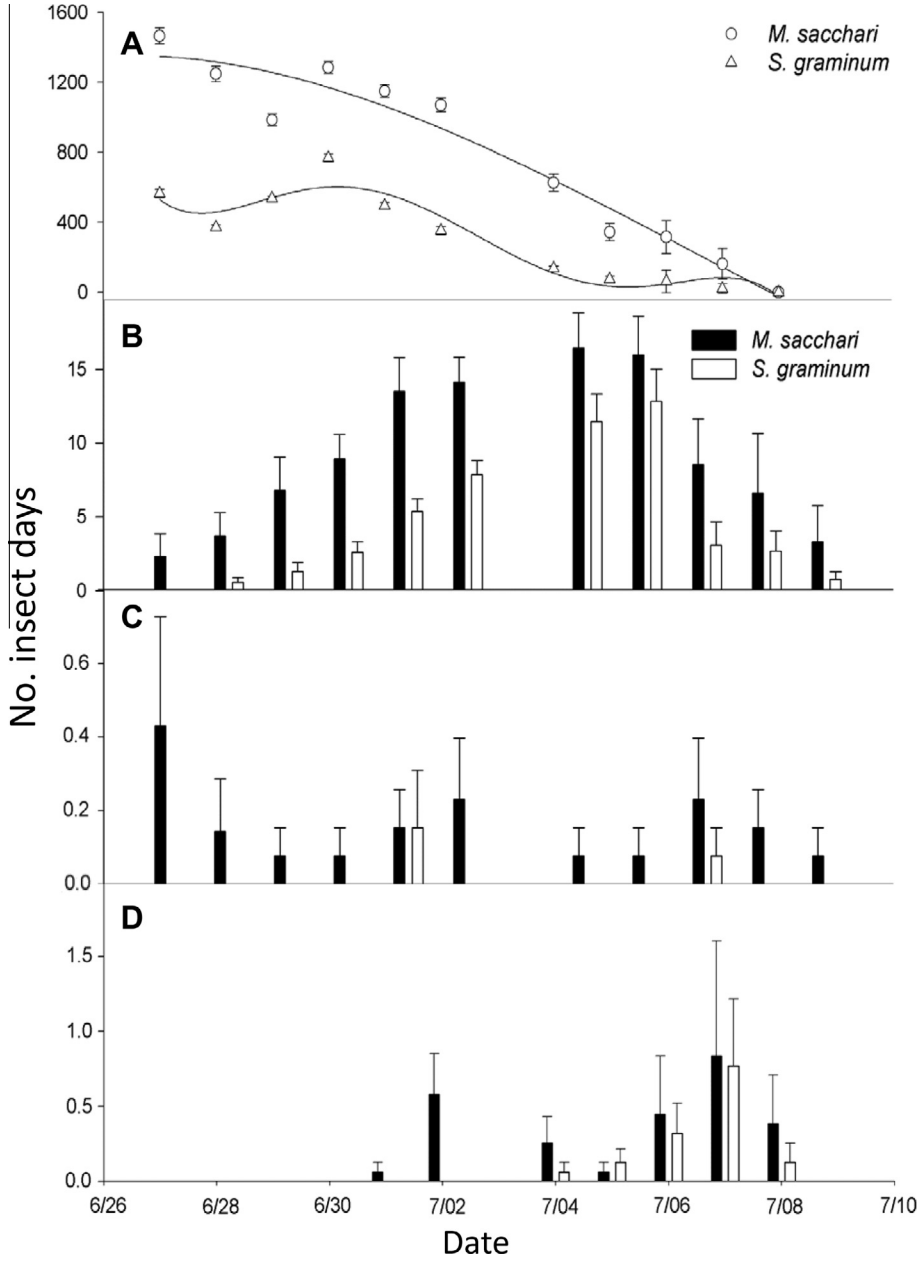

Fig. 1. Mean (+SE) daily counts of aphids (A), syrphid larvae (B), chrysopid larvae (C) and aphelinid mummies (D) observed per microcosm (pot of four sorghum plants infested with either *Melanaphis sacchari* or *Schizaphis graminum*), per sampling date, during the first cohort. *M. sacharri*: $Y = 0.6X^3 - 17.8X^2 + 18.4X + 1346.4$, $R^2 = 0.943$; *S. graminum*: $Y = -0.2X^5 + 8.2X^4 - 95.6X^3 + 470.2X^2 - 930.1X + 1079.5$, $R^2 = 0.920$.

Of the 125 Coccinellid adults observed in both cohorts, 91.9% were *Hippodamia convergens* Guerin-Meneville, 4.1% were *Coccinella septempunctata* L., 3.3% were *Coleomegilla maculata* DeGeer and 0.8% were *Harmonia axyridis* Pallas. Microcosms of *S. graminum* and *M. sacchari* did not differ in numbers of coccinellid adult days ($F = 0.93$; df = 1,26; $P = 0.344$), but there was a (borderline significant) tendency for *S. graminum* microcosms to register more anthocorid adult days ($Z = 1.89$; $P = 0.059$). Although *M. sacchari* microcosms registered almost twice as many chrysopid egg days as did *S. graminum* microcosms ($F = 6.63$; df = 1,26; $P = 0.016$), the number of chrysopid larval days did not differ between aphid species ($Z = 1.18$; $P = 0.240$). Parasitism by *Aphelinus* sp. (Hymenoptera: Aphelinidae) was observed in both *S. graminum* and *M. sacchari* colonies, with no difference between incidence of mummies in the first cohort ($F = 0.04$; df = 1,24; $P = 0.516$), but with significantly more on greenbug colonies in the second one ($Z = 0.98$; $P = 0.329$). Mummies of *Lysiphlebus testaceipes* (Cresson) (Hymenoptera: Aphidiidae) were observed only on greenbug microcosms in both cohorts.

There were several minor precipitation events during each cohort (Fig. 3), but no weather severe enough to negatively impact aphid microcosms. Median ambient temperatures were slightly below seasonal norms, averaging 24.1 ± 0.8 °C in the first cohort and 26.4 ± 0.4 °C in the second, a significant difference ($Z = 2.30$; $P = 0.021$).

4. Discussion

Despite being less than one kilometer apart, the two cohorts were placed in dramatically different habitats. The site of the first cohort was a habitat of much greater plant diversity, with nearby

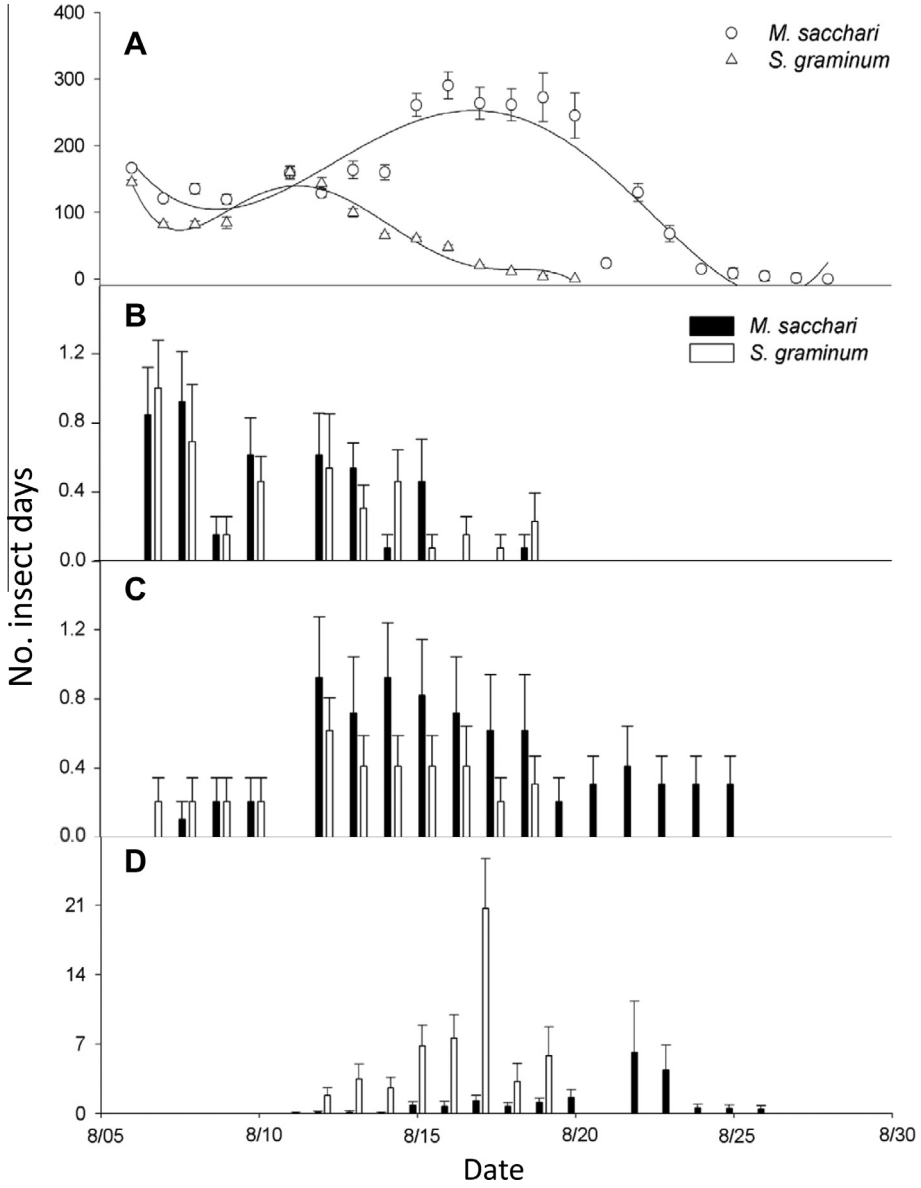

Fig. 2. Mean (±SE) daily counts of aphids (A), coccinellid adults (B), chrysopid larvae (C) and aphelinid mummies (D) observed per microcosm (pot of four sorghum plants infested with either *Melanaphis sacchari* or *Schizaphis graminum*), per sampling date, during the second cohort. *M. sacharri*: $Y = 6.5X^5 - 5.6X^4 - 0.8X^3 + 16.6X^2 - 89.8X + 249.5$, $R^2 = 0.832$; *S. graminum*: $Y = -0.02X^5 + 0.86X^4 - 13.66X^3 + 94.34X^2 - 265.48X + 329.41$, $R^2 = 0.947$.

trees and flowers that were absent from the site of the second cohort. Another difference was that pots in the first cohort functioned as virtually isolated microcosms in open space, whereas in the second, they blended into a virtually continuous canopy of similar plants. Thus, the first cohort provided better foraging conditions for visually searching predators, and the second, greater opportunity for larval predators to arrive from (or disperse to) adjacent plants. It could be argued that some differences between cohorts were driven by seasonal changes in insect abundance because the two sets of observations were made in different time frames. However, all observations were made within a two month period of very similar, midsummer weather conditions, apart from slightly warmer median temperature in the second cohort. Thus, we infer that most of the clear-cut differences in insect observations between cohorts reflect local habitat effects rather than seasonal differences in abundance or activity levels. In comparison to the first cohort, the second lacked aphid flies, *Leucopis* sp., and velvet mites, *Erythraeus aphidivorous* Sundic (Sundic et al., 2015), only a single adult brown lacewing (Hemerobiidae) was observed, and syrphid abundance was orders of magnitude lower. The brown lacewing is sometimes found preying on aphids in wheat fields, but is also an arboreal species that exploits scales and other soft-bodied Hemiptera infesting cedars and pines (JPM, unpublished observations).

Microcosms of *M. sacchari* and *S. graminum* survived for similar periods in the first cohort, but those of *M. sacchari* survived about a week longer in the second (Figs. 1A and 2A). However, no microcosm of either species was successful in producing a generation of migrant alate (the occasional alate nymph was noted) and no serious plant damage occurred, so biological control of microcosms was successful for both aphid species in both cohorts. In the first cohort, microcosms of *M. sacchari* had about twice as many aphids as those of *S. graminum* on the first observation date, which we considered indicative of higher reproduction by the former species during the 72 h infestation period; this was taken into account in preparation of the second cohort and similar numbers of aphids

Table 1
Summary of total natural enemy occurrence on two cohorts of two aphid species (*Schizaphis graminum* and *Melanaphis sacchari*) established on sorghum plants in microcosms (pots each containing four plants) and observed daily until aphid colonies were eliminated. Values are total numbers of arthropod-days, by life stage, observed on all microcosms of each aphid species. The two cohorts were observed in different locations over different time frames (see text for details) in Hays, KS, in the summer of 2014.

Natural enemies	Life stage	Cohort 1		Cohort 2	
		S. graminum (n = 13)	*M. sacchari* (n = 13)	*S. graminum* (n = 14)	*M. sacchari* (n = 14)
Anthocoridae	Adults	0	0	39	11
	Nymphs	0	0	104	22
Aphelinidae	Adults	0	4	8	4
	Mummies	22	41	771	319
Aphidiidae	Adults	2	5	0	0
	Mummies	47	0	225	0
Chamaemyiidae	Larvae	0	24	0	0
Chrysopidae	Adults	3	12	10	34
	Eggs	194	609	204	401
	Larvae	3	19	35	97
Coccinellidae	Adults	0	3	58	64
	Egg masses	0	8	2	97
	Larvae	0	77	2	24
Erythraeidae	Nymphs	12	5	0	0
Hemerobiidae	Adults	0	0	0	1
	Eggs	0	181	0	0
Nabidae	Adults	0	1	3	2
Syrphidae	Adults	5	9	0	0
	Eggs	1308	1626	13	37
	Larvae	631	1278	33	60
	Pupae	0	5	0	1

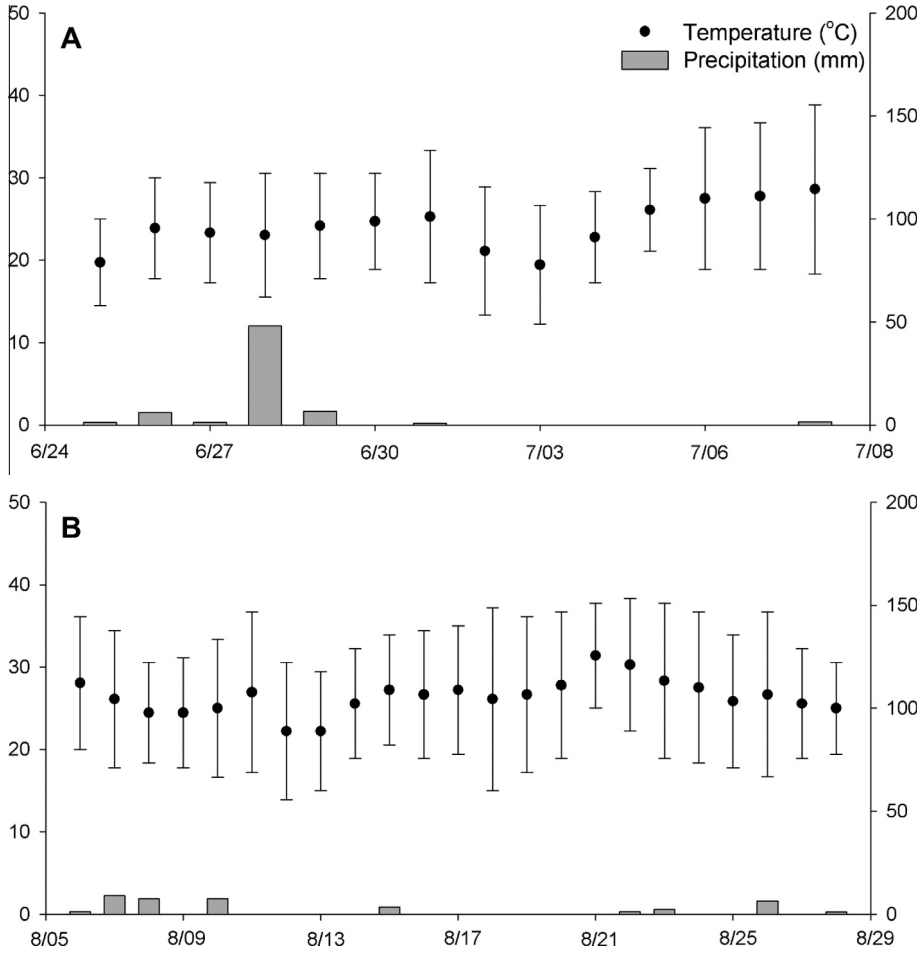

Fig. 3. Median (±maximum and minimum) daily temperatures and precipitation during the first (A) and second (B) aphid cohorts.

per microcosm were obtained for the two species on the first observation date. Laboratory observations indicated that the reproductive rate of *M. sacchari* at 23.5 °C was double that of *S. graminum* during the first week of adult life (FC, unpublished data); 4–5 nymphs per female, per day, as compared to 2–3 for greenbug. Because aphid reproductive rate tends to increase with

temperature, the higher median temperature that prevailed during the second cohort may have further enhanced the reproductive advantage of *M. sacchari* relative to *S. graminum* and contributed to its higher peak abundance.

Adult syrphids are known to respond to specific host plants as well as aphid species (Almohamad et al., 2007), and their abundance in the first cohort clearly demonstrates good responses by *A. obliqua* to both aphid species infesting sorghum. The greater abundance of aphids in microcosms of *M. sacchari* compared to *S. graminum* throughout the first cohort may account for the higher levels of oviposition by both syrphids and chrysopids on the former. For example, oviposition by syrphid species on lettuce infested with *Nasonovia ribis-nigri* (Mosely) scales with aphid colony size (Nelson et al., 2012). Syrphids are proficient in discovering aphid colonies in their earliest stages of development, often before they are large enough to attract other predators (e.g., Kan, 1988a,b). When syrphid larval counts were expressed as a percentage of egg observations, proportionally fewer larvae than expected were observed on *S. graminum* microcosms (48.2% versus 78.5%, Table 1). Syrphid larvae are notorious for egg cannibalism (e.g., Branquart et al., 1997; Belliure and Michaud, 2001) and lower aphid densities in *S. graminum* microcosms relative to those of *M. sacchari* may have led to higher rates of egg cannibalism.

Notably, syrphid eggs and larvae were orders of magnitude lower in abundance in our second cohort compared to the first. Adult syrphids depend on flowers for nectar to fuel flight and pollen to mature eggs (Haslett, 1989; Bugg et al., 2008), resources that were readily available in the locality of the first cohort, but absent from the vicinity of the second. For example, Meyer et al. (2009) found syrphid density strongly affected by the amount of pollen and nectar locally available for adults, as well as the presence of aphid-infested plants for larvae. Syrphids are notably rare in most field studies assessing aphid mortality in High Plains cereal crops (e.g., Rice and Wilde, 1988; Nechols and Harvey, 1998), very likely because of the scarcity of essential resources for adult females in large scale cereal monocultures with aggressive weed control. Another factor may be physical differences in habitat structure between the cohorts (microcosms in the open versus within a closed canopy); syrphids use both visual and chemical cues in locating aphid colonies and thus tend to prefer foraging in open habitats. For example, Michaud and Browning (1999) found six times as many syrphid eggs on colonies of brown citrus aphid, *Toxoptera citricida* (Kirkaldy), infesting heavily pruned citrus trees in open habitat compared to those infesting adjacent trees with dense canopies.

Chrysopids were the only major predator group to appear in similar numbers in both cohorts. Observations of chrysopid larvae were relatively few in consideration of the numbers of eggs observed. Larval chyrsopids are also notorious cannibals (e.g., Duelli, 1981; Bar and Gerling, 1985; Mochizuki et al., 2006) and some emigration from microcosms may have occurred. However, larval observations in the first cohort corresponded to 1.5% and 3.1% of egg observations on *S. graminum* and *M. sacchari* microcosms, respectively, compared to 17.2% and 24.2% in the second cohort, where larval dispersal should have been easier. Consequently, we suspect more intraguild predation on chrysopid eggs and/or young larvae occurred in the first cohort. Larvae of *A. obliqua* were the dominant predators in the first cohort and thus prime suspects as the IG predator; they were the first predator to oviposit and their eggs hatched very quickly. Similarly, larvae of a common European species, *Episyrphus balteatus* DeGeer (Diptera: Syrphidae) have been reported as intraguild predators of *C. carnea* larvae (Hindayana et al., 2001).

Based on prior observations, we expected coccinellids to be the primary source of aphid mortality in both cohorts, but this was true only in the second one. In central Kansas, adult coccinellids emerge from hibernation in early spring and have a single generation on aphids in wheat. The resulting adults leave the maturing wheat and enter reproductive diapause, which facilitates their survival through summer months when aphids are scarce (Michaud and Qureshi, 2006). To break diapause, female *H. convergens* require ad libitum access to greenbugs for three to four days, but our aphid colonies did not appear to be sufficiently large to retain adult beetles within microcosms for such a period. Adults were quick to colonize the second cohort and rapidly inflicted heavy aphid mortality, likely reducing aphid numbers during the first 24 h before the first observations were made. These beetles were directly observed consuming the aphids, but abandoned the plants as aphid densities declined, without laying eggs and without completely eliminating the aphids (Fig. 2B), permitting some resurgence in aphid numbers (Fig. 2A). The greater resurgence of *M. sacchari* in the second cohort, despite being initially reduced to similar densities as *S. graminum*, likely reflects its higher reproductive rate.

All immature coccinellids recorded in the first cohort resulted from a single female *H. axyridis* Pallas that laid two egg masses totaling 89 eggs on plants in one microcosm. This species is largely arboreal, as evidenced by its contributions to aphid biological control in pine trees (McClure, 1987), pecans (Tedders and Schaefer, 1994), apple (Brown and Miller, 1998), and citrus (Michaud, 1999) and is not normally associated with cereal aphids, except in the vicinity of trees, as in this case. The virtual absence of *H. convergens* from the first cohort (only one adult observed) may have been a location effect – substantial distance from potential reservoirs of *H. convergens* – combined with early discovery of the aphids by other predator groups, especially syrphids. Coccinellid females are known to avoid oviposition on plants contaminated by conspecific larval residues (Ruzicka, 2002), heterospecific larval residues (Ruzicka, 2006), and even those of other aphidophagous insects (Ruzicka, 2001), including possibly syrphid larvae (Alhmedi et al., 2010). Although avoidance of syrphid larvae and their residues has not been shown for *H. convergens*, females of this species avoid oviposition on plants contaminated with residues of conspecific larvae and those of *C. maculata* (Michaud and Jyoti, 2007). It is also possible that voracious feeding by syrphids quickly reduced aphid numbers below levels sufficient to attract beetles from any distance or retain those that did arrive.

Aphelinid and aphidiid parasitoids were observed in both aphid cohorts, but only *Aphelinus* sp. mummies formed on *M. sacchari*, although both occurred on *S. graminum*. In laboratory trials, we found that the two aphid species were attacked equally by female *L. testaceipes*, but no parasitoid larvae developed in any of the *M. sacchari* nymphs that were stung ($n > 100$). Two trials were conducted with *L. testaceipes* sourced from two different collections. Subsequently, two separate DNA extractions from the aphids tested positive for *Hamiltonella defensa* (C. Vorburger, pers. comm.), a secondary endosymbiont known to protect aphids from parasitism by aphidiid wasps (Vorburger et al., 2009). Genotype x genotype interactions exist between strains of *H. defensa* and those of aphidiid parasitoids, resulting in varying levels of protection in infected aphids (Rouchet and Vorburger, 2012). We conclude that this particular *H. defensa* strain is highly effective in protecting *M. sacchari* against our local strain of *L. testaceipes*, and is thus could be an impediment to biological control in regions where this wasp is a key mortality factor controlling aphids in sorghum, probably anywhere south of the Kansas–Oklahoma border (Jones et al., 2007). However, *H. defensa* can be acquired and lost among aphid clones, and parasitoid populations can evolve to overcome the resistance of particular strains (Rouchet and Vorburger, 2014), so it will be important to determine if infected clones are widely distributed, and if virulence against *H. defensa* exists in *L. testaceipes* populations. Complicating matters is the fact that the parasitoids

themselves serve as vectors of the symbiont (Gehrer and Vorburger, 2012). Mummies of *L. testaceipes* are common on *M. sacchari* in south Texas and appear to have normal emergence (R. Villanueva, pers. comm.), so infection with *H. defensa* does not appear to be universal among invasive clones of *M. sacchari* in the USA.

Some comment is warranted on differences in detectability among the various natural enemy species, and among life stages, within the sampling regime employed. Each microcosm was examined by two people for about 15 min daily, sufficient time to approximate aphid numbers and count natural enemies with reasonable accuracy. Sessile life stages (e.g., eggs, mummies, etc.) and larval stages that never leave aphid colonies (i.e., syrphids) have high detectability in this protocol, whereas more motile and/or secretive larvae (e.g., chrysopids and hemerobiids) are likely to be less detectable. Similarly, any predators that were exclusively nocturnal foragers would not have been detected. It was also not possible to sample any insects hiding deep in the whorl of the plant without doing so destructively, so this was done only on the last sampling date. Adult predators are highly active and may not spend much longer on an aphid colony than it takes to oviposit, reducing their detectability relative to immature stages. Adult syrphids and chrysopids do not consume aphids and eggs provide reliable evidence of their presence, so the issue is of little consequence for these groups. However, the actual numbers of adult *H. convergens* arriving at microcosms in the second cohort would have been substantially underestimated in 15 min of observation and oviposition by females does not occur prior to 3–4 days of aphid consumption. We inferred that our microcosms in the second cohort were not large enough, nor abundant enough, to withstand the functional response of *H. convergens* females, or cause them to break their reproductive diapause.

We conclude that all the major groups of aphidophagous species inhabiting High Plains cereal crops are preadapted to respond to, and exploit, sugarcane aphid, although the higher reproductive rate of *M. sacharri* relative to *S. graminum* may render conservation biological control of the former species more problematic. The identification and incorporation of plant resistance traits into sorghum cultivars that diminish the aphid's reproductive rate would therefore be a valuable approach to complement biological control of *M. sacchari*. The results of this study suggest that the ability of indigenous aphidophagous guilds to respond to, and ultimately control, invasive aphid species may be often underestimated, which would explain why effective biological control of invasive aphids typically evolves in time, even when classical programs fail to establish exotic natural enemies.

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